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Improved Biotyping Schemes for *Campylobacter jejuni* and *Campylobacter coli*

R. MARTIN ROOP II,[1] ROBERT M. SMIBERT,[2] AND NOEL R. KRIEG[1]*

Departments of Biology (Microbiology Section)[1] and Anaerobic Microbiology,[2] Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

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***Campylobacter jejuni* (20 strains) and *Campylobacter coli* (12 strains) were assigned to four biovars for each species based on phenotypic tests that were easy to perform and interpret. The resulting biotyping schemes offer a greater degree of distinction among *C. jejuni* and *C. coli* strains than any of the other biotyping schemes previously described for these organisms.**

Campylobacter jejuni is a major cause of gastroenteritis in humans (4, 20) and is currently being isolated as frequently as *Salmonella* and *Shigella* species (2, 3). *Campylobacter coli* also produces human enteric disease but is not encountered as frequently as *C. jejuni* (14, 21). Although *C. jejuni* and *C. coli* have many phenotypic characteristics in common, DNA homology studies have shown that they are separate species (1, 8, 11, 12, 15, 16, 19, 25). The host range for these two organisms is also different: *C. coli* is isolated primarily from pigs and poultry (14) and occasionally from dogs (19), whereas *C. jejuni* can be isolated from cattle, sheep, dogs, cats, poultry, and other animals (5, 14). For this reason, accurate identification to the species level is necessary for studying the epidemiology of campylobacter-associated gastroenteritis. Identification of a strain as belonging to a particular biovar (biotype) or serotype is also very useful in epidemiological studies. There are several serotyping schemes that have been developed for these organisms (11, 13, 17) and they have proven very useful, but the reagents for these schemes are not commercially available, and a recent report indicates that identification to the species level by serotyping is not always accurate (9).

To date, three schemes have been proposed for biotyping *C. jejuni* and *C. coli* strains (10, 22; H. Lior, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C279, p. 358). One is useful only for *C. jejuni* (22), and another does not differentiate between *C. jejuni* and *C. coli* strains (10). Lior's scheme can be applied to *C. jejuni* or *C. coli* strains. This report describes new schemes for biotyping both *C. jejuni* and *C. coli* strains that are simple to use and easy to interpret.

The campylobacter strains used in this study were isolated from a variety of animals (Table 1) and identified to the species level by both their phenotypic characteristics and high DNA homology (>60%) with their type strains (18).

Alkaline phosphatase activity was measured by using a modification of the technique described by Hébert et al. (10). The top 1 to 2 ml of growth from two cultures grown in semisolid brucella medium for 48 h was used to inoculate a Roux bottle containing 200 ml of brucella agar supplemented with 0.025% each of ferrous sulfate, sodium bisulfite, and sodium pyruvate (FBP agar) (6). The agar was overlaid with 50 ml of brucella broth supplemented with 0.05% ferrous sulfate and sodium pyruvate and 0.025% sodium bisulfite (FBP broth) (6). Roux bottles were incubated aerobically at 37°C for 24 h. Cells were harvested at 12,000 × *g* in a refrigerated centrifuge and washed once with 0.85% sterile saline. The cells were suspended in 0.85% saline and adjusted to a turbidity equivalent to a no. 2 McFarland standard. The cell suspension (0.5 ml) was dispensed into a test tube (16 by 125 mm), and 0.5 ml of alkaline phosphatase reagent (0.2% *p*-nitrophenylphosphate dissolved in 0.005 M glycine–5×10^{-4} M $MgCl_2$ [pH 10.5]) (10) was added. The test was incubated for 6 h at 37°C. Any yellow color was considered to be a positive reaction. *C. jejuni* 13136 served as a positive control, and sterile saline was the negative control. FBP agar plates supplemented with 0.2% DNA and 0.005% methyl green (24) were used to detect DNase activity. Growth from a 48-h-old semisolid culture was used as the inoculum, and plates were incubated at 37°C under an atmosphere of 6% O_2, 5% CO_2, and 89% N_2 for 72 h. A zone of clearing greater than 3 mm (total zone size) around the area of growth was considered to be a positive reaction. Hippurate hydrolysis was determined by the method of Harvey (7). The ability of strains to grow in a minimal medium (MM) was tested in a semisolid MM composed of (grams per liter): glutamic acid, 2.0; proline, 1.2; aspartic acid, 0.7; leucine, 0.7; niacin, 0.01; $(NH_4)_2SO_4$, 3.0; K_2HPO_4, 4.0; NaCl, 0.05; $Na_2S_2O_3 \cdot 5H_2O$, 0.8; Na_2CO_3, 0.4; $MgSO_4 \cdot 7H_2O$, 0.24; $FeSO_4 \cdot 7H_2O$, 0.012; and agar, 1.6. The pH was adjusted to 7.0 with KOH. MM is a modification of a medium described previously by Smibert (23). Two drops from a Pasteur pipette of a culture grown in semisolid brucella medium for 24 to 48 h was used to inoculate 5 ml of MM in a tube (16 by 125 mm). Cultures were examined for growth at 24, 48, and 72 h. Semisolid brucella medium was used as a positive control. A pellicle of growth similar to that occurring in semisolid brucella medium was considered to be a positive reaction. A thin haze or no growth was considered to be negative. In questionable cases, a serial transfer was made to fresh MM; only those strains showing growth after transfer were considered to be positive. All of the above tests were done in triplicate. Any test yielding questionable results was repeated.

The 20 *C. jejuni* strains used in this study could be assigned to four biovars based on alkaline phosphatase and DNase activity and on hippurate hydrolysis (Table 1). Hippurate hydrolysis is an important characteristic for identifying *C. jejuni*, but an occasional strain will be hippurate negative (8, 9, 18). Other characteristics can be used to differentiate *C. jejuni* from *C. coli* (Table 2). It is important to properly identify these organisms to the species level before assigning biovar designations. Altogether, 75% of the human strains used in this study belonged to biovar 2, which was also represented by a bovine and an ovine strain. It

* Corresponding author.

TABLE 1. Biotyping schemes for *C. jejuni* and *C. coli* strains and assignment of strains used in this study

Organism	Biovar	Alkaline phosphatase activity	DNase activity	Hippurate hydrolysis	Strain and source[a]
C. jejuni	1	+	+	+	B7619; human feces
					4164A; human feces
					11151; human blood
					13136; ovine fetus
					N82-10A; ovine fetus
					N82-11A; ovine fetus
					61-784; chicken
	2	+	−	+	ATCC 33560; bovine feces
					H840; human feces
					12019; human blood
					12375; human blood
					82214; human blood
					Holy Cross; human blood
					12191; human blood
					11641B76; human blood
					ATCC 33250; human blood
					ATCC 33251; human blood
					Veit; ovine fetus
	3	+	+	−	H325; human feces
	4	−	−	+	PC 340; unknown
C. coli	1	+	+	+	ATCC 33559; porcine feces
					C607; porcine feces
					C602; porcine feces
					541; porcine feces
					H550; human feces
					7292B76; human blood
					76-3227; human blood
					C679; chicken feces
	2	+	−	+	C699; chicken feces
	3	−	−	+	80-15573;porcine feces
					81-3592-3; porcine feces
	4	−	−	−	80-1184-2; porcine feces

[a] Further information regarding the origin of the strains has been presented by Roop et al. (18).

would be interesting to determine whether this is the predominant biovar among strains of *C. jejuni* isolated from poultry, because poultry has been suggested to be an important source of human campylobacter infections (21). Unfortunately, only one strain of *C. jejuni* from poultry was available for use in this study, and it belonged to biovar 1.

Alkaline phosphatase and DNase activity and the ability to grow in MM were used to place 12 *C. coli* strains into four biovars (Table 1). An occasional strain of *C. coli* may fail to grow in MM, and this can be a useful trait for biotyping if the strain has been properly identified to the species level. All three human isolates used here belonged to biovar 1, which also included four pig isolates and a poultry isolate. Pork has been suggested to be a possible source of human infection in several European countries, and in these countries there is a higher rate of isolation of *C. coli* from human illness than there is in Britain or the United States (14, 21).

The biotyping scheme proposed by Skirrow and Benjamin (22) is useful only for *C. jejuni* strains and is based on the production of H_2S in an iron-based "sensitive" medium. All of the 20 strains used in this study were Skirrow biotype 1. The schemes proposed in this report separate *C. jejuni* into more biovars than does that of Skirrow and Benjamin and also provide a scheme that is useful for *C. coli*. Even though *C. coli* is not as prevalent in human disease as *C. jejuni*, it is a human pathogen, and epidemiological markers for *C. coli* would prove very useful in an outbreak of gastroenteritis caused by *C. coli*.

Lior (Abstr. Annu. Meet. Am. Soc. Microbiol. 1983) developed a biotyping scheme based on rapid H_2S production, DNase activity, and hippurate hydrolysis that is basically an expansion of that proposed by Skirrow and Benjamin (22). This scheme is useful for both *C. jejuni* and *C. coli*, but the number of potential biotypes for each species is limited to four for *C. jejuni* and two for *C. coli*. However, the schemes described in this report could be expanded to eight potential biovars for each species.

Hébert et al. (10) proposed a biotyping scheme for *C. jejuni* and *C. coli* strains based on DNase activity, hippurate hydrolysis, and growth on charcoal-yeast extract agar and have applied it to 123 human isolates. One problem with this scheme is that *C. jejuni* and *C. coli* strains are not differentiated. A biotyping scheme is most useful if the strains are properly identified to the species level before they are assigned to biovars. Another problem with this scheme is the use of charcoal-yeast extract agar, which is somewhat difficult to make; moreover, we found it difficult to determine growth responses on charcoal-yeast extract agar. In contrast, the MM used in the biotyping scheme described in this report for *C. coli* is easy to prepare, and the growth reactions are very simple to interpret.

TABLE 2. Characteristics useful for differentiating *C. jejuni* and *C. coli* strains[a]

Characteristic	*C. jejuni*	*C. coli*
Hippurate hydrolysis	+	−
Growth in MM	−	+
H_2S production, triple sugar iron slant	−	+[b]

[a] Further information regarding the tests for determining these characteristics has been presented by Roop et al. (18).

[b] Triple sugar iron slants were freshly prepared, and only those that had water of syneresis were used. This water of syneresis was essential to demonstrate H_2S production. Slants were incubated at 37°C under an atmosphere of 6% O_2, 5% CO_2, and 89% N_2 for 7 days. *C. coli* strains produced H_2S at the junction of the butt and slant (18).

C. jejuni and, to a lesser extent, *C. coli* are important causes of gastroenteritis in humans. The biotyping schemes described in this report consist of phenotypic tests that are easy to perform and interpret. They provide good separation of strains within each species and should be very useful for epidemiological studies of *C. jejuni* and *C. coli* infections. They would allow clinical laboratories that are currently unable to perform serotyping to obtain preliminary epidemiological information. In addition, if biotyping and serotyping are combined, this would provide even more specific epidemiological information about these strains than either technique can alone.

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